

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

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In re Application of
Masayuki FURUYA, et al.
Serial No. 10/518,640
Filed: December 20, 2004
For: PROCESS FOR PRODUCING GLYCOSIDE

Group Art Unit: 1623

Examiner: Ganapathy Krishnan

Honorable Commissioner of Patents and Trademarks
United States Patent and Trademark Office
Washington, D. C. 20231

Sir:

DECLARATION UNDER 37 CFR 1.132

I, Akinori NAGATOMO, declare and state that:

1. In March 1990, I finished Master Course in Peptide Science, Department of Chemistry of Faculty of Science in Kyushu University.

Since April 1990, I have been an employee of MITSUI TOATSU CHEMICALS Inc., which has been named MITSUI Chemicals, Inc. Until now, I have been assigned to Fine & Performance Chemicals Laboratory and engaged in the research work concerning material science.

I am a co-inventor of the invention described in the above-identified application, and have a full understanding of the present invention.

2. I carried out the following experiment in order to demonstrate an advantage in preparing a diglycoside (a glycoside linked to two of sugars) by the reaction under the condition that acetic acid produced as a byproduct is removed at a specific rate and temperature to maintain the concentration of acetic acid in the reaction system at a low level.

Experiment

Experiment 1

To a reactor were fed 36.8 g (0.2 mol) of gallic acid methyl ester (hereinafter referred to as "GM"), 226.4 g (0.58 mol) of pentaacetyl- β -D-glucose (hereinafter referred to as " β -PAG"), and 530 g of mixed xylenes, and then 4.4 g (15 mole percent relative to the GM) of boron trifluoride diethyl etherate was added. The reaction was performed at a temperature of 43°C to 47°C under a pressure of 4.0 kPa to 3.6 kPa (30 mmHg to 27 mmHg) for 16 hours. During the reaction, 2.0 g of boron trifluoride diethyl etherate was further added. A distillate was successively collected. The total weight of the distillate was 440 g after 16 hours. The distillate contained 20.8 g of acetic acid. During the reaction, 150 g and 73 g of the mixed xylenes were further added at 6 and 12 hours, respectively, after starting the reaction. After finishing the reaction, the analysis of the resulting reaction mixture indicated that the yield of gallic acid methyl ester-bis(2,3,4,6-tetra-O-acetylglucoside) was 91.5% based on the GM. The resulting reaction mass was cooled to room temperature to form crystals and the pressure in the reactor was increased to atmospheric pressure. The crystals were filtered and washed twice

with 56 g of xylene, followed by drying, to give 155.6 g of 3,5-bis(2,3,4,6-tetra-O-acetylglucosyloxy)gallic acid methyl ester. The purity and corrected yield based on the purity were 94.5% and 87.1%, respectively. The concentration of the acetic acid was 0.32 percent to 0.38 percent by weight during the reaction.

Experiment 2

To a reactor were fed 18.4 g (0.1 mol) of GM, 156.1 g (0.40 mol) of β -PAG, and 300 g of mixed xylenes, and then 14.7 g (100 mole percent relative to the GM) of boron trifluoride diethyl etherate was added. The reaction was performed at 80°C. Since the reaction did not proceed at six hours after starting the reaction, the reaction was finished at eight hours after starting the reaction. The yield of gallic acid methyl ester-3,5-bis(2,3,4,6-tetra-O-acetylglucoside) was 19.0 %. The reaction mixture contained 49% (relative to the GM) of gallic acid-3-(2,3,4,6-tetra-O-acetylglucoside), and no GM was detected. Further, plural peaks each derived from unknown object were observed. The concentration of acetic acid was 1.3 percent to 1.5 percent by weight in the reaction system during the reaction.

Experiment 3

To a reactor were fed 18.4 g (0.1 mol) of GM, 156.1 g (0.40 mol) of β -PAG, and 300 g of mixed xylenes, and then 14.7 g (100 mole percent relative to the GM) of boron trifluoride diethyl etherate was added. The reaction was performed at 25°C for 18 hours. The yield of gallic acid methyl ester-3,5-bis(2,3,4,6-tetra-O-acetylglucoside) was 49.0 %. The

reaction mixture contained 51% (relative to the GM) of gallic acid-3-(2,3,4,6-tetra-O-acetylglucoside), and no GM was detected. The concentration of acetic acid was 1.6 percent to 1.8 percent by weight in the reaction system during the reaction.

Experiment 4

To a reactor were fed 11.03 g (0.1 mol) of hydroquinone (hereinafter referred to as "HQ"), 17.1 g (0.3 mol) of β -PAG, and 150 g of mixed xylenes, and then 2.1 g (15 mole percent relative to the HQ) of boron trifluoride diethyl etherate was added. The reaction was performed at a temperature of 43°C to 47°C under a pressure of 4.0 kPa to 3.6 kPa (30 mmHg to 27 mmHg) for 12 hours. The analysis of the reaction mixture indicated as follows: The yields of hydroquinone-1,4-bis(2,3,4,5-tetra-O-acetylglucoside), and hydroquinone-mono(2,3,4,5-tetra-O-acetylglucoside) were 89.0% and 10.0% relative to the HQ, respectively. Unreacted HQ content was 1.0%. The concentration of acetic acid was 0.45 percent to 0.50 percent by weight in the reaction system during the reaction.

Experiment 5

To the reactor were fed 39.0 g (0.1 mol) of β -PAG, 16.5 g (0.15 mol) of HQ, 70 g of diethylene glycol dibutyl ether and 0.5 g of p-toluenesulfonic acid. The reaction was performed under the reduced pressure at 15 mmHg, at 110°C for four or eight hours. After washing with water at ambient temperatures, 140 g of heptane was added thereto to precipitate a crystal. The crystal was subjected to recrystallization in ethyl alcohol solvent. Then, 31.7 g of tetraacetylarbutin, that is,

hydroquinone-mono(2,3,4,5-tetra-O-acetylglucoside) was obtained from either of the reaction system of the different reaction time (yield: 72 %).

3. From the results of the above Experiments and based on my best knowledge and experience on glycosylation, I conclude that:

Comparing with Experiments 1 to 3, in preparing a diglycoside, the reaction under the condition that acetic acid produced as a byproduct is removed at a specific rate and temperature to maintain the concentration of acetic acid in the reaction system at a low level is important on the yield, that is, 91.5 % for Experiment 1, 19.0 % for Experiment 2 and 49.0 % for Experiment 3.

Referring to Experiments 1 and 4, the method as presently claimed is applicable to a gallic acid derivative as well as a hydroquinone derivative, in both systems a diglycoside was obtained in high yield.

Comparing with Experiments 4 and 5, the process of Experiment 5 is suitable for obtaining a mono-glycoside, not for a diglycoside. On the other hand, the process of Experiment 4 is suitable for obtaining a diglycoside.

Accordingly, it is believed that Experiments 1 to 5 demonstrate an advantage in preparing a diglycoside by the reaction under the condition that acetic acid produced as a byproduct is removed at a specific rate and temperature to maintain the concentration of acetic acid in the reaction system at a low level.

The undersigned declares further that all statements made herein of our own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

This day of July 07, 2008

Akinori Nagatomo
Akinori NAGATOMO